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(54) **Use of blends of mannich acrylamide polymers and dimethyldiallylammonium halide polymers for flocculating enzyme broth streams.**

(57) Blends of Mannich acrylamide polymers and dimethyldiallylammonium halide polymers have been found to be superior flocculants for enzyme broth streams yielding higher solid compaction and greater supernatant clarities than the use of either polymer alone.

**EP 0 448 926 A2**

# USE OF BLENDS OF MANNICH ACRYLAMIDE POLYMERS AND DIMETHYLDIALLYLAMMONIUM HALIDE POLYMERS FOR FLOCCULATING ENZYME BROTH STREAMS

## Background of the Invention

The production of enzymes by fermentation has been carried out for many years. Fermentation is usually carried out in stainless steel equipment i.e. mixing and blending tanks, and seed and main fermentators. Constant temperature, automatic foam and pH controllers and air purifiers are employed since the absence of foreign microorganisms is essential. Tap water is generally combined with the media ingredients and enzyme recovery begins as soon as fermentation is terminated. The medium is cooled and centrifuges are used to remove bacteria and large insolubles from the supernatant followed by filters to separate smaller particles. Enzyme is concentrated and removed from the filtrate by the addition of a precipitating agent. The precipitate is then further treated by additional filtering and drying etc. and is then standardized such as by using sodium chloride.

Proteases are enzymes which have been found to be particularly useful in industrial areas including cheese making, meat tenderizing, bread baking, beer haze elementation, digestive aid preparations, garment cleaning, pharmaceutical preparation and the like. Those proteases produced by cultivation can be used as food additives.

Characteristic of the protease enzyme broth is the formation of a suspension that does not settle. Upon centrifugation of a sample in a test tube, solids will be deposited in the lower 70% of the test tube and only the upper 30% of the tube will be clear supernatant solution.

One of the most difficult problems involving enzyme production is the isolation of the enzyme from its broth. Although many flocculating agents have been used for the precipitation of enzyme broths, most have suffered from some disability which renders the agent less attractive commercially. Examples of flocculants used commercially include epichlorohydrin-dimethylamine condensation products cross-linked with diethylenetriamine/dicyanamide; Mannich acrylamide polymers and polydimethyldiallylammonium halides. These additives, although tolerable, oftentimes fail to result in the isolation of the enzyme sufficiently e.g. the solids are not compacted; the supernatant has poor clarity, etc. Thus, the search for more effective flocculants is continuing and the discovery of useful materials which do not suffer from the deficiencies of the existing commercial flocculants would satisfy a long felt industrial need.

## Summary Of The Invention

The present invention relates to a process for precipitating aqueous enzyme broths comprising using, as the flocculating agent, a blend of a Mannich acrylamide polymer and a diallyldimethylammonium halide polymer, which blend has been found to provide more effective flocculation of precipitate than either of these known flocculants alone.

## Description Of The Invention Including Preferred Embodiments

This invention relates to a process for precipitating an aqueous enzyme broth which comprises adding to said broth a flocculant comprising a blend of 1) a Mannich acrylamide polymer and 2) a dimethyldiallylammonium halide polymer.

The blends are composed of the two polymers 1); 2) in a ratio of 3:1 to 1:30, by weight, real polymer solids, respectively, preferably 1:1.5 to 1:7, respectively.

The Mannich acrylamide polymers are generally well known in the art, examples thereof being disclosed in U.S. Patent No. 4,137,164, hereby incorporated herein by reference. Generally, these polymers are homopolymers of acrylamide or copolymers thereof with such commoners as acrylonitrile, methacrylamide, acrylic acid etc. in amounts up to about 50%, preferably 5-50% of the resultant copolymer. The polymers have molecular weights ranging from about 10,000 to about 3,000,000 and are chemically modified to provide dimethylaminomethyl groups to the extent that the polymer contains 25-100 mol percent of these groups, preferably at least 40 mol percent.

The dimethyldiallylammonium halide (DADM) polymers are likewise known in the art, examples thereof being disclosed in U.S. Patent No. 4,092,467, hereby incorporated herein by reference. These polymers are homopolymers of DADM or copolymers thereof with such monomers as acrylamide, vinyl pyrrolidone, etc. in amounts up to about 20% of the resultant polymer. These polymers have Intrinsic Viscosities ranging from about 0.1-4.00 deciliters per gram. The halide can be chloride, fluoride, bromide or iodide.

The polymer blend may be added to the enzyme broth as such or the two polymers may be added individually but as near the same time as possible, since the enhanced benefit of the polymers is attributed to their presence in the broth coincidentally. The amount of the blend added to the broth is that effective to produce the clearest supernatant and achieve the highest solid compaction as possible. Generally, amounts ranging from about 10 to 100 grams of polymer blend per liter of broth, preferably from about 25-75 grams per liter, is effective, although higher or lower amounts may be useful in specific instances.

The following examples are set forth for purposes of illustration only and are not to be construed as limitations on the present invention. Products A and B are set forth in the following tables, with respect to the amounts employed, as 0.065% aqueous polymer solutions while Product C is expressed as a 20.0% aqueous polymer solution. Clarity is measured by UV absorbance at 660 microns.

In the following examples, the Mannich acrylamide polymers employed are each Mannich polyacrylamide of 70% aminomethylation and are further designated as follows:

<u>Product</u>	<u>Percent Solids</u>	<u>Brookfield Viscosity-cps</u>
A	5.9-6.4%	26,000-34,000
B	5.5-6.1	34,000-46,000

The dimethyldiallylammonium halide polymer is polydimethyldiallylammonium chloride further designated as follows:

<u>Product</u>	<u>Percent Solids</u>	<u>Intrinsic Viscosity-cps</u>
C	19.5-20.5	2.0-3.5

In order to test the effectiveness of various polymers in flocculating enzyme broths, the following test procedure is utilized: To 5ml of broth in a 15ml clinical, graduated centrifuge tube are added 5ml. samples of various concentrations of the polymer solutions. Each sample is mixed by inverting the stoppered clinical tube 20 times, the clinical tube is then centrifuged for 5 minutes and the volume of the compacted enzyme is visually measured. The lower the value, the better. In addition, the clarity of the supernatant is measured by UV absorbance at 660 microns. A value of 0.3-0.4% is acceptable and below 0.1 is superior.

Table I is a measure of the effect of single polymer flocculants on the precipitation of enzyme broths. It is a comparative table showing that although Products A, B and/or C individually may perform effectively with regard to compaction (% volume solids) or clarity, the flocculants alone fail to perform satisfactorily as regards both criteria.

TABLE I  
Effect of Single Polymer Flocculants on The Flocculation of Enzyme Broth

Exp. No.	Flocculant	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
1	None	-	-	72	poor
	C 1st run	5	20	28	1.58
		10	20	27	0.622
		15	20	26	0.477
		20	20	26	0.438
		25	20	27	0.393
2	C 2nd run	10	20	28	-
		10	20	29	0.879
		15	20	24	0.338
		20	20	26	0.306
		25	20	26	0.245
3	C 3rd run	5	20	28	1.14
		10	20	27	0.432
		15	20	24	0.303
		20	20	24	0.272
		25	20	25	0.251
4	C 4th run	5	20	31	0.649
		10	20	31	0.577
		15	20	31	0.299
		20	20	31	0.240
		25	20	31	0.232

TABLE I (Cont'd)

Exp. No.	Flocculant Product	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
5	A	15 25 35	20 20 20	- 18 18	- 1.72 0.272
6	A	15 17.5 20	20 20 20	20 20 19	0.253 0.225 0.154
7	B 1st run	20 30 40	20 20 20	15 15 17	- 0.116 0.258
8	B 2nd run	15 25 35	20 20 20	- 18 18	- 1.68 0.253
9	B	15 17.5 20	20 20 20	21 22 20	0.796 0.298 0.184

Table II reflects the unexpectedly superior result achieved when using blends of C and B polymers on an enzyme broth. As can be seen, in this instance, as the blend approaches a 1/3 mixture, the compaction and the clarity are drastically improved.

TABLE II  
Improved Compaction and Clarity of  
Supernatant with Polymer Blends

<u>Exp.</u> <u>No.</u>	<u>Flocculant</u>	<u>Ratio</u>	<u>g/l</u>	<u>No. of</u> <u>Tube</u> <u>Inversions</u>	<u>Centrifuge</u> <u>5 Minutes</u> <u>% Volume</u> <u>Solids</u>	<u>Clarity of</u> <u>Supernatant</u> <u>660 Microns</u>
10	C		15 20 25	20 20 20	23 23 24	0.384 0.309 0.319
11	B		20 30 40	20 20 20	15 15 17	Poor 0.116 0.258
12	B/C	1.3/1	20 30 40	20 20 20	18 17 18	0.588 0.270 0.614
13	B/C	1/3	15 20 25	20 20 20	17 19 19	0.218 0.101 0.093

Examples 14-20 of the Table III represent comparative results as in Table II using Product A instead of Product B. As can be seen, the blends result in clarities superior to either polymer alone. The compaction values are not as good for the blends as Product A alone; however, the excellent overall results achieved by the blends are clearly shown.

In Examples 21-26 varying ratios of Product B to Product C are shown to be excellent as the level of

Polymer B increases, especially with regard to the compaction. Example 24, at 35 g/l results in the outstanding value of 0.069 with a compaction value of 20.

The blends of A/C and B/C (Examples 27-32) show exceptional clarity in conjunction with acceptable compaction, compare Example 9.

5        Examples 33-43 form further support for the unexpected synergistic results achieved by the blends in that compaction falls well within the accepted range and increasingly superior clarity at dosage of 10-20 g/l are set forth.

      An enzyme fermentation broth is treated in Examples 44-52 with the benefits of the polymer blends being clearly evident. Examples 53-62 reflect the same inventive trend.

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TABLE III  
Polymer Blend Ratios  
vs.  
Effect on Compaction and Supernatant Clarity

<u>Exp. No.</u>	<u>Flocculant</u>	<u>Ratio</u>	<u>g/l</u>	<u>No. of Tube Inversions</u>	<u>Centrifuge 5 Minutes % Volume Solids</u>	<u>Clarity of Supernatant 560 Microns</u>
14	C	-	15 25 35	20 20 20	30 28 29	0.824 0.465 0.359
15	A	-	15 25 35	20 20 20	- 18 18	- 1.72 0.275
16	A/C	3/1	15 25 35	20 20 20	18 20 20	0.609 0.629 0.128
17	A/C	1/1.5	15 25 35	20 20 20	20 20 20	0.955 0.230 0.190
18	A/C	1/3	15 25 35	20 20 20	20 20 20	0.472 0.172 0.131
19	A/C	1/7	15 25 35	20 20 20	20 22 23	0.263 0.153 0.150

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
20	A/C	1/30	15 25 35	20 20 20	24 24 25	0.290 0.166 0.208
21	B	-	15 25 35	20 20 20	- 18 18	- 1.680 0.253
22	B/C	3/1	15 25 35	20 20 20	20 19 19	0.017 0.672 0.152
23	B/C	1/1.5	15 25 35	20 20 20	18 20 20	1.530 0.198 0.114
24	B/C	1/3	15 25 35	20 20 20	20 20 20	0.314 0.260 0.069
25	B/C	1/7	15 25 35	20 20 20	20 20 23	0.306 0.103 0.172
26	B/C	1/30	15 25 35	20 20 20	24 24 25	0.299 0.125 0.298

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
27	A/C	3/1	10 15 20 25	20 20 20 20	20 22 19 20	0.479 0.098 0.119 0.069
28	A/C	1/3	10 12.5 15 17.5 20	20 20 20 20 20	23 21 20 20 20	0.176 0.058 0.070 0.034 0.053
29	A/C	1/30	15 20 25	20 20 20	25 23 23	0.046 0.031 0.039
30	B/C	3/1	10 12.5 15 17.5 20	20 20 20 20 20	- - - - -	- 0.484 0.194 0.135 0.095
31	B/C	1/3	10 12.5 15 17.5 20	20 20 20 20 20	- 20 19 19 20	0.253 0.124 0.106 0.063 0.066

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
32	B/C	1/30	10 12.5 15 17.5 20	20 20 20 20 20	24 23 22 23 23	0.138 0.073 0.056 0.043 0.031
33	C	-	10 15 17.5 20.0 22.5 25	20 20 20 20 20 20	34 31 31 30 30 30	0.563 0.340 0.319 0.290 0.095 0.103
34	A	-	15 17.5 20	20 20 20	20 20 19	0.253 0.225 0.154
35	A/C	3/1	10 12.5 15 17.5 20	20 20 20 20 20	23 21 21 21 22	0.932 0.244 0.202 0.096 0.146

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
36	A/C	1.3/1	10 12.5 15 17.5 20	20 20 20 20 20	21 22 20 22 21	0.367 0.237 0.150 0.089 0.118
37	A/C	1/1.5	10 12.5 15 17.5 20	20 20 20 20 20	23 22 20 21 21	0.329 0.207 0.166 0.102 0.109
38	A/C	1/3	15 17.5 20	20 20 20	22 22 21	0.145 0.177 0.072
39	B	-	15 17.5 20	20 20 20	21 22 20	0.796 0.298 0.184
40	B/C	3/1	10 12.5 15 17.5 20	20 20 20 20 20	23 21 21 21 22	0.932 0.244 0.202 0.096 0.146

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
41	B/C	1.3/1	10 12.5 15 17.5	20 20 20 20	21 22 20 19	0.367 0.237 0.129
42	B/C	1/1.5	10 12.5 15 17.5 20	20 20 20 20 20	20 20 19 19 20	0.742 0.232 0.176 0.125 0.120
43	B/C	1/3	15 17.5 20	20 20 20	20 20 20	0.184 0.106 0.095
44	C	-	10 12.5 15 17.5 20 22.5	20 20 20 20 20 20	30 30 29 30 32 32	0.462 0.388 0.369 0.365 0.348 0.345

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
45	A/C	3/1	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	18 19 19 18 20 21	0.859 0.565 0.403 0.306 0.263 0.233
46	A/C	1.3/1	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	20 20 20 20 22 22	0.481 0.390 0.276 0.259 0.219 0.188
47	A/C	1/1.5	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	21 21 21 22 22 23	0.498 0.388 0.318 0.288 0.231 0.251
48	A/C	1/3	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	20 21 22 23 24 25	0.331 0.282 0.253 0.140 0.201 0.224

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
49	B/C	3/1	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	20 18 18 19 18 19	- 0.656 0.148 0.381 0.197 0.327
50	B/C	1.3/1	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	17 18 18 18 19 20	1.01 0.505 0.321 0.272 0.208 0.205
51	B/C	1/1.5	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	18 19 20 19 20 19	0.522 0.331 0.243 0.173 0.145 0.130
52	B/C	1/3	12.5 15 17.5 20 22.5 25	20 20 20 20 20 20	20 20 20 20 21 22	0.299 0.240 0.166 0.155 0.152 0.160

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
53	A/C	3/1	16	20	21	0.266
			18	20	22	0.303
			20	20	20	0.195
			22	20	20	0.345
			24	20	20.5	0.248
54	A/C	1/1.5	16	20	21.5	0.282
			18	20	22	0.296
			20	20	21	0.177
			22	20	20	0.209
			24	20	20.5	0.177
55	A/C	1/3	16	20	21	0.082
			18	20	22.5	0.065
			20	20	22	0.090
			22	20	21	0.054
			24	20	22	0.031
56	A/C	1/7	16	20	23	0.222
			18	20	23	0.058
			20	20	22.5	0.016
			22	20	23	0.032
			24	20	24	0.010

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
57	A/C	1/30	16	20	26	0.290
			18	20	25	0.123
			20	20	25	0.077
			22	20	25	0.036
			24	20	25	0.032
58	B/C	3/1	16	20	20	0.171
			18	20	20	0.145
			20	20	20	0.151
			22	20	20	0.117
			24	20	20	0.070
59	B/C	1/1.5	16	20	20	0.146
			18	20	20	0.120
			20	20	20	0.115
			22	20	20	0.113
			24	20	20	0.102
60	B/C	1/3	16	20	20	0.162
			18	20	20	0.103
			20	20	20	0.095
			22	20	19.5	0.093
			24	20	19.5	0.074

TABLE III (Cont'd)

Exp. No.	Flocculant	Ratio	g/l	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
61	B/C	1/7	16	20	20.5	-
			18	20	20.5	0.037
			20	20	20	0.039
			22	20	21.5	0.041
			24	20	21.5	0.034
62	B/C	1/30	16	20	25	0.283
			18	20	24	0.102
			20	20	24	0.100
			22	20	25	0.059
			24	20	24	0.029

Table IV reflects the results of increasing the polymer blend dosage rate in Examples 63-72. As can be seen, upon treating an enzyme fermentation broth, increased blend dosages results in magnificent clarity values as low as 0.018 although compaction values are somewhat sacrificed.

In Examples 73-81, an enzyme broth is treated and at rather low dosage rates, the combined compaction/clarity values are considered acceptable.

TABLE IV  
Polymer Blend Effect on Compaction and Supernatant  
Clarity on an Enzyme Broth

<u>Exp. No.</u>	<u>Flocculant</u>	<u>Ratio</u>	<u>g/l Flocculant</u>	<u>No. of Tube Inversions</u>	<u>Centrifuge 5 Minutes &amp; Volume Solids</u>	<u>Clarity of Supernatant 660 Microns</u>
63	A/C	3/1	- 40 50	- 20 20	- 32 30	- -- 0.506
64	A/C	1/1.5	30 40 50	20 20 20	30 30 -	1.300 0.088 -
65	A/C	1/3	30 40 50	20 20 20	- 30 31	- 0.140 0.096
66	A/C	1/7	- 40 50	- 20 20	- 30 30	- 0.074 0.040
67	A/C	1/3	- 40 50	- 20 20	- 30 28	- 0.056 0.018
68	B/C	3/1	40 50	20 20	28 30	- 0.291

TABLE IV (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
69	B/C	1/1.5	40 50	20 20	31 29	1.580 0.172
70	B/C	1/3	40 50	20 20	30 30	0.280 0.052
71	B/C	1/7	40 50	20 20	30 30	0.142 0.044
72	B/C	1/30	30 40 50	20 20 20	30 30 -	0.063 0.027 -
73	C	-	10 12.5 15 17.5 20 22.5	20 20 20 20 20 20	54 48 45 45 37 35	- - - 1.00 0.042 0.032
74	A/C	1/3	22.5 25 27.5	20 20 20	32 30 30	- 0.258 0.178

TABLE IV (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
75	A/C	1/7	15 20 22.5 25 27.5	20 20 20 20 20	33 29 28 28 26	0.375 0.156 0.178 0.089 0.105
76	A/C	1/11.5	15 20 22.5 25 27.5	20 20 20 20 20	30 28 27 27 27	0.154 0.169 0.199 0.091 0.126
77	A/C	1/30	15 20 22.5 25 27.5	20 20 20 20 20	30 28 27 27 27	0.124 0.189 0.265 0.127 0.141
78	B/C	1/3	22.5 25 27.5	20 20 20	29 30 30	- - -

TABLE IV (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
79	B/C	1/7	15 20 22.5 25 27.5	20 20 20 20 20	31 31 31 28 28	- - 0.493 0.227 0.168
80	B/C	1/11.5	15 20 22.5 25 27.5	20 20 20 20 20	30 31 30 26 28	- 0.235 0.152 0.113 0.116
81	B/C	1/30	15 20 22.5 25 27.5	20 20 20 20 20	30 28 26 25 26	0.161 0.147 0.187 0.085 0.136

The effects of mixing are shown in Table V. An enzyme broth is treated with the blends, the clinical test tube being inverted from 10 to 100 times in Examples 82 and 83. As can be seen, undue agitation appears to deleteriously affect mechanical break-up of flocculated particles, leading to the creation of many fines. The same trend can be seen in Examples 84-89, in Examples 90-95 and in Examples 96-107.

TABLE V

Mixing Factor vs. Compaction and Supernatant Clarity

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
82	A/C	1.3/1	20	10	21	0.162
			20	20	20	0.195
			20	30	20	0.201
			20	50	20	0.284
			20	75	20	0.363
			20	100	20	0.397
83	B/C	1.3/1	20	10	23	0.143
			20	20	21	0.225
			20	30	20	0.179
			20	50	18	0.222
			20	75	20	0.347
			20	100	20	0.534
84	A/C	1/1.5	16	20	21	0.198
			18	20	21.5	0.085
			20	20	21	0.079
			22	20	20	0.058
			24	20	20.5	0.047
85	A/C	1/1.5	16	40	22	0.374
			18	40	20.5	0.229
			20	40	21	0.078
			22	40	21	0.102
			24	40	20	0.074

TABLE V (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
86	A/C	1/1.5	16 18 20 22 24	75 75 75 75 75	22 24 22 22 22	0.358 0.441 0.655 0.400 0.293
87	B/C	1/1.5	16 18 20 22 24	20 20 20 20 20	21 20.5 20 20.5 20	0.529 0.360 0.464 0.365 0.435
88	B/C	1/1.5	16 18 20 22 24	40 40 40 40 40	20 20 19.5 20 19.5	0.233 0.095 0.078 0.102 0.107
89	B/C	1/1.5	16 18 20 22 24	75 75 75 75 75	20.5 19.5 21 20.5 20	0.404 0.338 0.147 0.351 0.101

TABLE V (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of " Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
90	A/C	1/1.5	16 18 20 22 24	20 20 20 20 20	22.5 23 23.5 24 24	0.301 0.164 0.125 0.114 0.094
91	A/C	1/1.5	16 18 20 22 24	40 40 40 40 40	24 23 22.5 22.5 22.5	0.335 0.318 0.220 0.112 0.059
92	A/C	1/1.5	16 18 20 22 24	75 75 75 75 75	22.5 22.5 23 22.5 22.5	1.503 1.426 0.834 0.280 0.338
93	B/C	1/1.5	16 18 20 22 24	20 20 20 20 20	22 22.5 23 24 23	0.795 0.219 0.184 0.153 0.145

TABLE V (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
94	B/C	1/1.5	16 18 20 22 24	40 40 40 40 40	21.5 22 22 22.5 22	0.696 0.377 0.248 0.208 0.147
95	B/C	1/1.5	16 18 20 22 24	75 75 75 75 75	20 20.5 22.5 20.5 22	- 1.927 1.504 1.303 0.808
96	A/C	3/1	16 18 20 22 24	40 40 40 40 40	22.5 22.5 22 22 22.5	0.663 0.661 0.314 0.064 0.216
97	A/C	3/1	16 18 20 22 24	75 75 75 75 75	22.5 21.5 23 21 22.5	1.18 0.823 0.598 0.570 0.107

TABLE V (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
98	A/C	1/1.5	16 18 20 22 24	40 40 40 40	23 22 23.5 21 22	0.515 0.420 0.210 0.077 0.159
99	A/C	1/1.5	16 18 20 22 24	75 75 75 75 75	23.5 22.5 22 22 22.0	0.608 0.586 0.366 0.295 0.084
100	A/C	1/3	16 18 20 22 24	40 40 40 40 40	23 22.5 25 25 25	0.293 0.183 0.087 0.071 0.042
101	A/C	1/3	16 18 20 22 24	75 75 75 75 75	23 25 24 25 25	0.285 0.252 0.117 0.179 0.063

TABLE V (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
102	B/C	3/1	16 18 20 22 24	40 40 40 40 40	22 20 21 20 22	0.551 0.310 0.152 0.088 0.093
103	B/C	3/1	16 18 20 22 24	75 75 75 75 75	20 21 21 19.5	0.538 0.295 0.223 0.120
104	B/C	1/1.5	16 18 20 22 24	40 40 40 40 40	22 20.5 20 20 21.5	0.275 0.088 0.065 0.038 0.052
105	B/C	1/1.5	16 18 20 22 24	75 75 75 75 75	21.5 21.5 21 20 22	0.058 0.064 0.143 0.098 0.095

TABLE V (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
106	B/C	1/3	16 18 20 22 24	40 40 40 40	21.5 20.5 22 22 22.5	0.069 0.051 0.042 0.046 0.059
107	B/C	1/3	16 18 20 22 24	75 75 75 75 75	22 22 23 22.5 22.5	0.338 0.256 0.055 0.079 0.052

The broad effects of the blends of polymers of the present invention on an enzyme broth is shown in Table VI. The overall trend again supports the unique results achieved by said blends.

TABLE VI

Polymer Blend Ratios vs. Affect on Compaction  
and Clarity of Supernatant at 40 Inversions

<u>Exp. No.</u>	<u>Flocculant</u>	<u>Ratio</u>	<u>g/l Flocculant</u>	<u>No. of Tube Inversions</u>	<u>Centrifuge 5 Minutes &amp; Volume Solids</u>	<u>Clarity of Supernatant 660 Microns</u>
108	A/C	3/1	16 18 20 22 24	40 40 40 40 40	22 23 22.5 22 22	0.406 0.290 0.189 0.141 0.182
109	A/C	1/1.5	16 18 20 22 24	40 40 40 40 40	24 23 22.5 22.5 22.5	0.335 0.318 0.220 0.112 0.059
110	A/C	1/3	16 18 20 22 24	40 40 40 40 40	24.5 24 24.5 25 25.5	0.119 0.115 0.082 0.162 0.207
111	A/C	1/7	16 18 20 22 24	40 40 40 40 40	25 25.5 26 27 28.5	0.259 0.064 0.118 0.106 0.215

TABLE VI (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
112	A/C	1/30	16 18 20 22 24	40 40 40 40 40	29 29 29 30 30	0.123 0.101 0.106 0.104 0.066
113	B/C	3/1	16 18 20 22 24	40 40 40 40 40	20 20.5 20 20 20	0.860 0.482 0.356 0.301 0.235
114	B/C	1/1.5	16 18 20 22 24	40 40 40 40 40	21.5 22 22 22.5 22	0.696 0.377 0.248 0.208 0.147
115	B/C	1/3	16 18 20 22 24	40 40 40 40 40	22 21 22.5 22 22	0.057 0.096 0.062 0.066 0.110

TABLE VI (Cont'd).

Exp. No.	Flocculant	Ratio	g/l Flocculant	No. of Tube Inversions	Centrifuge 5 Minutes & Volume Solids	Clarity of Supernatant 660 Microns
116	B/C	1/7	16	40	23.5	0.078
			18	40	23.5	0.071
			20	40	24.5	0.068
			22	40	25	0.062
			24	40	25	0.019
117	B/C	1/30	16	40	28.5	0.172
			18	40	29	0.185
			20	40	29.5	0.169
			22	40	29.5	0.157
			24	40	30.5	0.145

## Claims

1. A process for the flocculation of an aqueous enzyme broth which comprises adding to said broth a flocculant comprising a mixture of 1) a Mannich acrylamide polymer and 2) a dimethyldiallylammonium halide polymer.

2. A process according to Claim 1 wherein the ratio of 1) to 2) ranges from about 3:1 to about 1:30, by weight, respectively.
3. A process according to Claim 1 wherein the ratio of 1) to 2) ranges from about 1:1.5 to about 1:7, by weight, respectively.
4. A process according to Claim 1 wherein 1) is a Mannich homopolymer of acrylamide.
5. A process according to Claim 1 wherein 1) is a Mannich copolymer of acrylamide containing 5-50% of a comonomer.
6. A process according to Claim 1 wherein 1) contains 25-100 mol percent of dimethylaminomethyl groups.
7. A process according to Claim 1 wherein 2) is a chloride.
8. A process according to Claim 1 wherein 2) is polydimethyldiallylammonium chloride.
9. A process according to Claim 1 wherein the enzyme is a protease.
10. A process according to Claim 1 wherein from about 10 to 100 grams per liter of broth of polymer blend is added.



(19)



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(54) **Use of blends of mannich acrylamide polymers and dimethyldiallylammonium halide polymers for flocculating enzyme broth streams.**

(57) Blends of Mannich acrylamide polymers and dimethyldiallylammonium halide polymers have been found to be superior flocculants for enzyme broth streams yielding higher solid compaction and greater supernatant clarities than the use of either polymer alone.

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## EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT			EP 91100312.7
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	<u>US - A - 4 508 825</u> (KIM et al.) * Abstract * --	1, 9	C 12 N 9/00 C 12 N 9/50
A	PATENT ABSTRACTS OF JAPAN, unexamined applications, C field, vol. 13, no. 69, February 16, 1989 THE PATENT OFFICE GOVERNEMENT page 9 C 569 * Kokai-no. 63-258 607 (EBARA INFILCO) * --	1	
D, A	<u>US - A - 4 137 164</u> (COSCIA et al.) * Abstract * --	1	
D, A	<u>US - A - 4 092 467</u> (WELCHER et al.) * Abstract * ----	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 12 N B 01 D
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 25-11-1991	Examiner WOLF
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

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